BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01871.x
www.brjpharmacol.org

Themed Section: Secretin Family (Class B) G Protein-Coupled Receptors – from Molecular to Clinical Perspectives

International Union of Basic and Clinical Pharmacology Review

Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR Review 1

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Keywords
accessory proteins; agonists and antagonists; class B GPCR; PAC_1 receptor; PACAP; signal transduction; splice variant; VIP; $VPAC_1$ receptor; $VPAC_2$ receptor

Received
25 October 2011
Revised
4 January 2012
Accepted
5 January 2012

This is the first in a series of reviews written by committees of experts of the Nomenclature Committee of the International Union of Basic and Clinical Pharmacology (NC-IUPHAR). A listing of all articles in the series and the Nomenclature Reports from IUPHAR published in Pharmacological Reviews can be found at http://www.GuideToPharmacology.org. This website, created in a collaboration between the British Pharmacological Society (BPS) and the International Union of Basic and Clinical Pharmacology (IUPHAR), is intended to become a "one-stop shop" source of quantitative information on drug targets and the prescription medicines and experimental drugs that act on them. We hope that the Guide to Pharmacology will be useful for researchers and students in pharmacology and drug discovery and provide the general public with accurate information on the basic science underlying drug action.

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are members of a superfamily of structurally related peptide hormones that includes glucagon, glucagon-like peptides, secretin, gastric inhibitory peptide (GIP) and growth hormone-releasing hormone (GHRH). VIP and PACAP exert their actions through three GPCRs – PAC_1, $VPAC_1$ and $VPAC_2$ – belonging to class B (also referred to as class II, or secretin receptor-like GPCRs). This family comprises receptors for all peptides structurally related to VIP and PACAP, and also receptors for parathyroid hormone, corticotropin-releasing factor, calcitonin and related peptides. PAC_1 receptors are selective for PACAP, whereas $VPAC_1$ and $VPAC_2$ respond to both VIP and PACAP with high affinity. VIP and PACAP play diverse and important roles in the CNS, with functions in the control of circadian rhythms, learning and memory, anxiety and responses to stress and brain injury. Recent genetic studies also implicate the $VPAC_2$ receptor in susceptibility to schizophrenia and the PAC_1 receptor in post-traumatic stress disorder. In the periphery, VIP and PACAP play important roles in the control of immunity and inflammation, the

How to cite: Harmar AJ, Fahrenkrug J, Gozes I, Laburthe M, May V, Pisegna JR *et al.* (2012). Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR Review 1. Br J Pharmacol 166: 4–17.

control of pancreatic insulin secretion, the release of catecholamines from the adrenal medulla and as co-transmitters in autonomic and sensory neurons. This article, written by members of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) subcommittee on receptors for VIP and PACAP, confirms the existing nomenclature for these receptors and reviews our current understanding of their structure, pharmacology and functions and their likely physiological roles in health and disease. More detailed information has been incorporated into newly revised pages in the IUPHAR database (http://www.iuphar-db.org/DATABASE/FamilyMenuForward?familyId=67).

LINKED ARTICLES

This article is part of a themed section on Secretin Family (Class B) G Protein-Coupled Receptors. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.166.issue-1

Abbreviations

BNST, bed nucleus of the stria terminalis; EAE, experimental autoimmune encephalomyelitis; ECL, enterochromaffin-like; GHRH, growth hormone-releasing hormone; GIP, gastric inhibitory peptide; LTP, long-term potentiation; NC-IUPHAR, International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification; N-ted, N-terminal ectodomain; PACAP, pituitary adenylate cyclase-activating polypeptide; PHI, peptide histidine isoleucine; PHM, peptide histidine methionine; PHV, peptide histidine valine; RAMP, receptor activity-modifying protein; SCN, suprachiasmatic nuclei; Th1, T helper 1; Th2, T helper 2; VIP, vasoactive intestinal peptide

Links to online information in IUPHAR-DB and the BPS Guide to Receptors and Channels

IUPHAR database	PAC_1 receptor *(in GRAC)*	PHV
[^{125}I]VIP	PAC_1 receptor *(in IUPHAR-DB)*	Ro 25-1553
[^{125}I]PACAP-27	PAC_1 receptor splice variants	Ro 25-1392
[Ala^{11,22,28}]VIP	PACAP-27	VIP
[Lys^{15},Arg^{16},Leu^{27}]VIP(1-7)/GRF(8-27)-NH_2	PACAP-38	$VPAC_1$ receptor *(in GRAC)*
CRF_2 receptor *(in GRAC)*	PACAP(6-38)	$VPAC_1$ receptor *(in IUPHAR-DB)*
CRF_2 receptor *(in IUPHAR-DB)*	PG 97-269	$VPAC_2$ receptor *(in GRAC)*
M65	PHI	$VPAC_2$ receptor *(in IUPHAR-DB)*
maxadilan	PHM	

This table lists chemical names, words and phrases which, in the online version of this article, are hyperlinked to relevant entries in http://www.guidetopharmacology.org, the common portal for data from IUPHAR-DB (Sharman *et al.*, 2011) and the BPS Guide to Receptors and Channels (Alexander *et al.*, 2011).

The endogenous peptide ligands – PACAP, VIP, PHI/PHM and PHV

Vasoactive intestinal peptide (VIP) was first isolated from porcine intestine as a 28-amino acid peptide, conserved in sequence between most mammals, capable of inducing vasodilatation in the canine femoral artery (Said and Mutt, 1970; 1972) and has subsequently been shown to have many other actions as a neuroendocrine hormone, putative neurotransmitter and cytokine. In common with the precursors of many other neuroendocrine peptides, the VIP precursor polypeptide (prepro-VIP) contains sequences encoding several additional biologically active peptides (Figure 1), including peptide histidine isoleucine [PHI; found in non-human mammals (Tatemoto and Mutt, 1981)], peptide histidine methionine [PHM; the human equivalent of PHI (Itoh *et al.*, 1983)] and peptide histidine valine [PHV; a C-terminally extended form of PHI and PHM (Yiangou *et al.*, 1987)]. The presence of VIP and specific VIP binding sites in defined pathways in the brain indicate that it may play an important role in CNS function (Besson *et al.*, 1986; Martin *et al.*, 1987). VIP is now widely accepted as a co-transmitter, with nitric oxide and carbon monoxide, of nonadrenergic, noncholinergic relaxation of both vascular and nonvascular smooth muscle (Said and Rattan, 2004) and with acetylcholine in exocrine glands (Fahrenkrug, 1993). VIP may also promote neuronal survival (Brenneman and Eiden, 1986) and regulate glycogen metabolism in the cerebral cortex (Sorg and Magistretti, 1992). VIP stimulates prolactin secretion from the pituitary (Reichlin, 1988) and catecholamine release from the adrenal medulla (Malhotra *et al.*, 1988). In the immune system, VIP regulates T cell traffic and inhibits mitogen-activated proliferation of T cells by inhibiting IL-2 production (Ottaway, 1987). Other actions of VIP include stimulation of electrolyte secretion and protection against oxidant injury (Gozes and Brenneman, 1989; Said, 1991; 1996).

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first identified as a 38-amino acid peptide (PACAP-38) from ovine hypothalamus on the basis of its ability to stimulate cyclic AMP production in rat anterior pituitary cells in culture (Miyata *et al.*, 1989). Subsequently, a

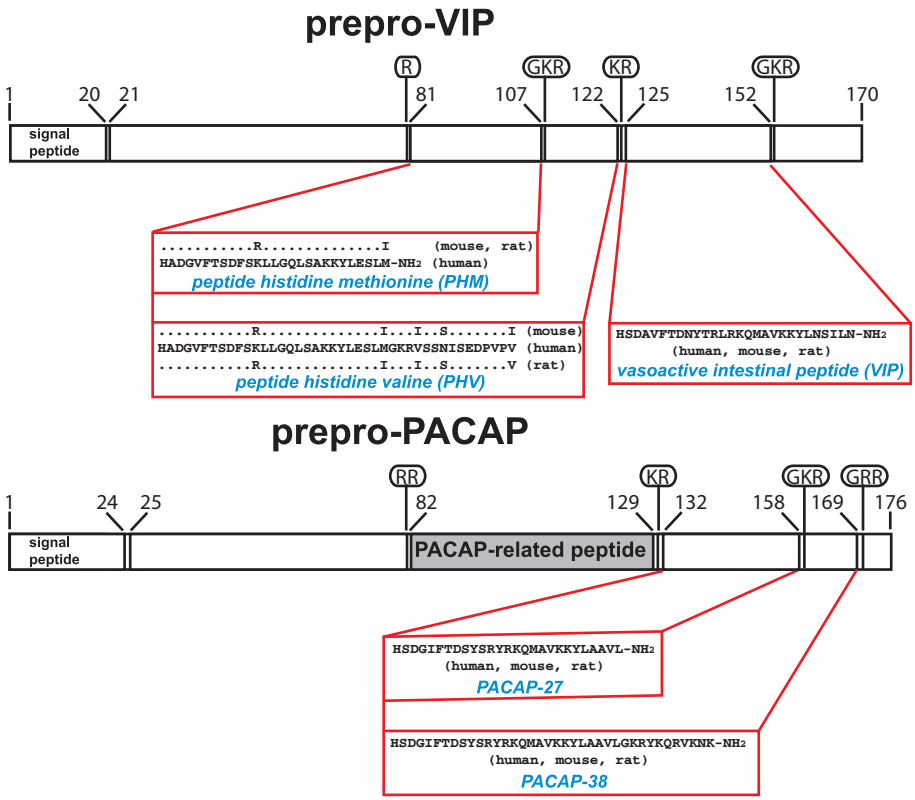

Figure 1

Structures of the precursors of VIP and PACAP and the biologically active peptides that they encode. Structures of the human VIP and PACAP precursors are shown, with sites of proteolytic processing (basic amino acids and glycine residues that donate the C-terminal amide groups of the mature peptides) indicated in ovals. Amino acid sequences of the human peptides and sequence variations in rat and mouse are given in single letter nomenclature. PACAP-related peptide displays sequence homology to PHM but has not been shown to be biologically active.

C-terminally truncated, 27-amino acid form of the peptide (PACAP-27) was isolated from the same source (Miyata *et al.*, 1990). The sequences of human, mouse, rat and sheep PACAP-38 are identical (Figure 1). In the CNS, PACAP and the mRNA encoding its precursor are most abundant in the hypothalamus, with lower levels in other brain regions (Ghatei *et al.*, 1993). PACAP is also present in peripheral tissues, such as the gastrointestinal tract, adrenal gland and testis (Ghatei *et al.*, 1993; Arimura and Shioda, 1995). PACAP is expressed in sympathetic neurons and in the cholinergic innervation of the adrenal medulla, where it is thought to facilitate secretion of catecholamines under conditions of high stress (Przywara *et al.*, 1996; Hamelink *et al.*, 2002). PACAP is also thought to regulate exocrine and endocrine secretion from the pancreas (Raufman *et al.*, 1991; Yada *et al.*, 1994). For a recent review of the structure and functions of PACAP and its receptors, see Vaudry *et al.* (2009).

The receptors: $VPAC_1$, $VPAC_2$ and PAC_1

Radioligand binding studies using [^{125}I]PACAP-27 (Shivers *et al.*, 1991) suggested the existence of two distinct receptors for PACAP in rat tissues, one with much greater affinity for PACAP than for VIP (the 'PACAP type I receptor') and a second with high affinity for both PACAP and VIP (the 'PACAP type II receptor'). Subsequently, two types of high-affinity VIP (PACAP type II) receptors were identified based on the relative potencies of natural and synthetic VIP analogues. In addition to the 'classical' VIP receptors from intestinal cells (Laburthe *et al.*, 1983), receptors with different pharmacology were identified in the human SUP-T1 lymphoblast cell line (Robberecht *et al.*, 1988) and in lung cancer cell lines (Luis and Said, 1990). Subsequently, two high-affinity receptors for both VIP and PACAP ('PACAP type II receptors') were cloned: the $VPAC_1$ receptor, first isolated from rat lung (Ishihara *et al.*, 1992) and the $VPAC_2$ receptor, first cloned from rat olfactory bulb (Lutz *et al.*, 1993). $VPAC_1$ and $VPAC_2$ receptors display comparable affinity for PACAP and VIP, whereas PACAP-27 and PACAP-38 are >100-fold more potent than VIP as agonists of most isoforms of the PAC_1 receptor.

The $VPAC_1$ receptor is widely distributed in the CNS, most abundantly in the cerebral cortex and hippocampus (Ishihara *et al.*, 1992; Usdin *et al.*, 1994), in peripheral tissues including liver, lung and intestine (Ishihara *et al.*, 1992; Usdin *et al.*, 1994; Ichikawa *et al.*, 1995; Sreedharan *et al.*, 1995; Kaltreider *et al.*, 1997; Reubi, 2000; Reubi *et al.*, 2000; Harmar *et al.*, 2004) and in T lymphocytes (Delgado *et al.*, 1996). In the CNS, the highest concentrations of messenger RNA encoding the $VPAC_2$ receptor are found in the thalamus and suprachiasmatic nucleus (SCN) and lower levels in the hippocampus, brainstem, spinal cord and dorsal root ganglia (Usdin *et al.*,

1994; Sheward *et al.*, 1995). The receptor is also present in many peripheral tissues, including smooth muscles in the cardiovascular, gastrointestinal and reproductive systems (Inagaki *et al.*, 1994; Usdin *et al.*, 1994; Adamou *et al.*, 1995; Krempels *et al.*, 1995; Wei and Mojsov, 1996; Reubi, 2000; Reubi *et al.*, 2000; Harmar *et al.*, 2004).

The 'PACAP type I receptor', which recognizes PACAP-27 and PACAP-38 with much higher potency than VIP (now referred to as PAC_1) was first identified in a rat pancreatic acinar carcinoma cell line (Pisegna and Wank, 1993). mRNA encoding this receptor is expressed predominantly in the CNS, most abundantly in the olfactory bulb, thalamus, hypothalamus, the dentate gyrus of the hippocampus and in granule cells of the cerebellum (Hashimoto *et al.*, 1996; Shioda *et al.*, 1997). The receptor is also highly expressed in the embryonic nervous system (Sheward *et al.*, 1998; Waschek *et al.*, 1998; Zhou *et al.*, 1999) and in a number of peripheral tissues, most abundantly in the adrenal medulla (Shivers *et al.*, 1991; Spengler *et al.*, 1993; Moller and Sundler, 1996; Reubi, 2000; Reubi *et al.*, 2000). There is apparent heterogeneity of PAC_1 receptors in tissues and cell lines, where two types of 'PACAP type I' pharmacology have been observed: type IA receptors, with high affinity for both PACAP-27 and PACAP-38; and type IB receptors, with high affinity for PACAP-38 but low affinity for PACAP-27 (Robberecht *et al.*, 1991; Shivers *et al.*, 1991). The difference between the two receptor subtypes may reflect differences in G protein coupling and second messenger mechanisms (Van Rampelbergh *et al.*, 1996) or result from alternative splicing of PAC_1 receptor mRNA.

Splice variants and accessory proteins

The diversity and functional consequences of alternative splicing events in class B GPCRs have been reviewed recently (Furness *et al.*, 2012). Splice variants differing in amino acid sequence in the extracellular N-terminal domain or the extracellular loops may display altered ligand affinity and selectivity, whereas splice variation in the intracellular loops or the C-terminus can influence signal transduction pathways. Although there is some evidence for the existence of splice variants of the $VPAC_1$ and $VPAC_2$ receptors, their functional importance is not yet clear (Dickson and Finlayson, 2009). In contrast, splice variation in the PAC_1 receptor is well established, complex and functionally important. Within the part of the PAC_1 receptor cDNA encoding the third intracellular loop, splice variants either containing or lacking each of two alternative exons (called 'hip' and 'hop' in rodents) exist. The hop exon exists in two forms (hop1 and hop2) as the result of the existence of two alternative splice acceptor sites three nucleotides apart. Thus, six possible splice variants, which differ in their intracellular signal transduction pathways, can be generated (Spengler *et al.*, 1993; Journot *et al.*, 1995). Four variants of the human PAC_1 receptor (null, SV-1, SV-2 and SV-3) resulting from alternative splicing of sequences equivalent to hip and hop1 have also been described (Pisegna and Wank, 1996) and were shown to differ in their ability to activate phospholipase C (PLC). In addition, splice variation in the N-terminal extracellular domain of the PAC_1 receptor has been reported. Splicing out of the 4th and 5th coding exons, leading to a 21 amino acid deletion, has been reported in human and mouse (Pantaloni *et al.*, 1996; Dautzenberg *et al.*, 1999). Surprisingly, the human splice variant, named 'PAC_1short' bound PACAP-27, PACAP-38 and VIP with similar high affinity and all three peptides stimulated cyclic AMP accumulation with similar potency (Dautzenberg *et al.*, 1999). Additional N-terminal splice variants resulting from splicing out of the 3rd, 4th and 5th exons of the human gene (Dautzenberg *et al.*, 1999) and by insertion of an additional 72 base pairs (exon 3a) encoding a sequence of 24 amino acids between coding exons 3 and 4 (Daniel *et al.*, 2001) have also been described.

Two class B receptors – the calcitonin receptor and the calcitonin receptor-like receptor – can form heteromers with members of a family of accessory proteins called RAMPs (receptor activity-modifying proteins 1, 2 and 3) to generate multiple distinct receptor types with different specificities for endogenous peptide ligands (Barwell *et al.*, 2012). The $VPAC_1$ receptor (but not $VPAC_2$ or PAC_1) has been shown to be able to interact with RAMPs; in this case ligand specificity is not altered but the $VPAC_1$ receptor-RAMP2 heteromer displays altered signal transduction specificity, with significant enhancement of agonist-mediated phosphoinositide hydrolysis with no change in cyclic AMP stimulation (Christopoulos *et al.*, 2003).

Although there is some evidence for the presence of PHI-selective receptors in mammalian tissues and the cloning of a PHI-selective receptor from the goldfish *Carassius auratus* has been reported (Tse *et al.*, 2002), there is no convincing evidence at present for a separate PHI receptor in mammals. However, it remains possible that such a receptor, either encoded by a novel gene, or resulting from alternative splicing of known genes, by interaction of known genes with accessory proteins, or through homo/hetero-oligomerization, may be discovered in the future.

Pharmacology

For recent critical reviews of the pharmacology and signalling properties of VIP and PACAP receptors, see Laburthe *et al.*, 2007; Dickson and Finlayson, 2009. Progress in characterizing the functions of the three receptor types has been hindered by the limited number of selective drugs available (Table 1). [$Ala^{11,22,28}$]VIP (Nicole *et al.*, 2000) and [Lys^{15},Arg^{16},Leu^{27}]VIP(1–7)/GRF(8–27)-NH_2 (frequently abbreviated as [K^{15},R^{16},L^{27}]VIP(1–7)/GRF(8–27) in the literature; Gourlet *et al.*, 1997b) are selective agonists of the $VPAC_1$ receptor and PG 97–269 is a selective antagonist (Gourlet *et al.*, 1997a). Ro 25–1392 (Xia *et al.*, 1997) is the most selective $VPAC_2$ agonist to date. There is no highly selective $VPAC_2$ antagonist as yet: PG99-465 (Moreno *et al.*, 2000) has been used as a selective $VPAC_2$ receptor antagonist in a number of physiological studies, but has been reported to be a partial agonist of $VPAC_2$ in some functional assays (EC_{50} = 5 nM) and act as a full agonist at $VPAC_1$ (EC_{50} = 8 nM) and PAC_1 (EC_{50} = 71 nM) receptors (Dickson *et al.*, 2006). The tissue distribution of $VPAC_1$ and $VPAC_2$ receptors can be determined by *in vitro* receptor autoradiography using [^{125}I]-VIP as the radioligand and displacement with the $VPAC_1$ selective agonist [Lys^{15},Arg^{16},Leu^{27}]VIP(1–7)/GRF(8–27)-NH_2 and the $VPAC_2$

Table 1

Useful pharmacological tools for the characterization of VIP and PACAP receptors

Receptor	$VPAC_1$	$VPAC_2$	PAC_1
Endogenous ligands	VIP (8.5–9.8) PACAP-27 (8.9) PACAP-38 (8.2) PHI (pIC_{50} = 6.0) PHM (5.7) PHV (pIC_{50} = 5.5)	VIP (7.8–8.8) PACAP-27 (7.6–8.0) PACAP-38 (pEC_{50} = 7.7–9.3) PHI (pIC_{50} = 7.5) PHV (pIC_{50} = 8.8)	VIP (6.0–6.3) PACAP-27 (8.5) PACAP-38 (8.8–9.0) Values for the 'PAC_1short' splice variant (Dautzenberg *et al.*, 1999) are as follows: VIP (8.4) PACAP-27 (8.5) PACAP-38 (8.8)
Selective agonists	[$Ala^{11,22,28}$]VIP (8.1: Nicole *et al.*, 2000) [Lys^{15},Arg^{16},Leu^{27}]VIP(1–7)/GRF(8–27)-NH_2 (pIC_{50} = 7.7–9.0: Gourlet *et al.*, 1997b)	Ro 25-1553 (8.0: Gourlet *et al.*, 1997b,c) Ro 25-1392 (8.0: Xia *et al.*, 1997)	Maxadilan (pEC50 = 9.2: Moro and Lerner, 1997)
Selective antagonists	PG97-269 (pIC_{50} = 7.1–8.7: Gourlet *et al.*, 1997a)	–	Max.d.4 (Tatsuno *et al.*, 2001) M65 (pIC_{50} = 6.6–6.8: Uchida *et al.*, 1998)
Radioligands	[^{125}I]-VIP, [^{125}I]-PACAP-27, [^{125}I]-Ro 25-1553	[^{125}I]-VIP, [^{125}I]-PACAP-27	[^{125}I]-PACAP-27

Affinity data from the IUPHAR Database (Sharman *et al.*, 2011) are shown in parentheses. Unless otherwise indicated, pK_i values determined in radioligand binding assays using the cloned human receptor are shown.

selective agonist Ro 25-1553 to distinguish the two receptor types (Reubi *et al.*, 2000; Harmar *et al.*, 2004). [^{125}I]-Ro 25-1553 can also be used to localize $VPAC_2$ receptors (Vertongen *et al.*, 1997; Reubi *et al.*, 2000; Harmar *et al.*, 2004).

The most selective agonist of PAC_1 receptors is maxadilan, a peptide isolated from the salivary glands of sand flies (*Lutzomyia longipalpis*), which has no sequence homology to VIP or PACAP (Moro and Lerner, 1997). Max.d.4 (maxadilan Δ24–42) and M65 (maxadilan Δ25–41; Uchida *et al.*, 1998) are synthetic variants of maxadilan, which display activity as PAC_1 antagonists but the use of these peptides has been limited due to problems with their availability. Finally, it is important to note that although PACAP(6–38) has been used as a PAC_1 receptor antagonist in many studies, it also exhibits high potency at $VPAC_2$ receptors (Dickinson *et al.*, 1997).

Receptor structure and signal transduction mechanisms

For all class B receptors, the large N-terminal ectodomain plays a crucial role in ligand recognition, prompting structural studies of this domain (Laburthe and Couvineau, 2002; Laburthe *et al.*, 2007; Couvineau and Laburthe, 2012a, b). As initially described for the mouse CRF_2 receptor (Grace *et al.*, 2004), the structure comprises a crucial sushi domain characterized by two antiparallel β sheets and stabilized by three disulphide bonds and a salt bridge sandwiched between aromatic rings of two tryptophan residues. Structures of the ectodomains of PAC_1 receptor (Sun *et al.*, 2007; Kumar *et al.*, 2011) and $VPAC_2$ receptors (PDB ID: 2X57) have been determined by X-ray or NMR and a structural model of the $VPAC_1$ receptor obtained by homology modelling associated with photoaffinity experiments (Tan *et al.*, 2006). Photoaffinity labelling has also demonstrated that the ectodomain of the PAC_1 receptor is the major binding site for PACAP (Dejda *et al.*, 2011). The data are consistent with the two-site model for peptide binding to class B GPCRs (Figure 2), in which the C-terminal and central α-helical parts of the peptide hormone interact with the sushi domain in the N-terminal ectodomain (N-ted) ultimately positioning the N-terminus of the peptide to contact the transmembrane region resulting in receptor activation (Laburthe and Couvineau, 2002; Laburthe *et al.*, 2007; Bourgault *et al.*, 2009; Couvineau and Laburthe, 2012b). This latter contact region remains elusive since no structure for a full-length class B GPCR has been determined yet. However, the presence of a helix N-capping motif in cognate peptide ligands of all class B receptors, including VIP and PACAP, supports that the folded backbone conformation of a N-cap is formed upon receptor binding and constitutes a key element underlying class B GPCR activation (Neumann *et al.*, 2008).

Functions of VIP and PACAP receptors in the CNS

The widespread distribution of VIP and PACAP and their receptors in the brain and periphery has led to many hypotheses concerning the physiological functions of these receptors. However, the availability of mutant mice lacking VIP (Colwell *et al.*, 2003), PACAP (Kawaguchi *et al.*, 2003; Colwell

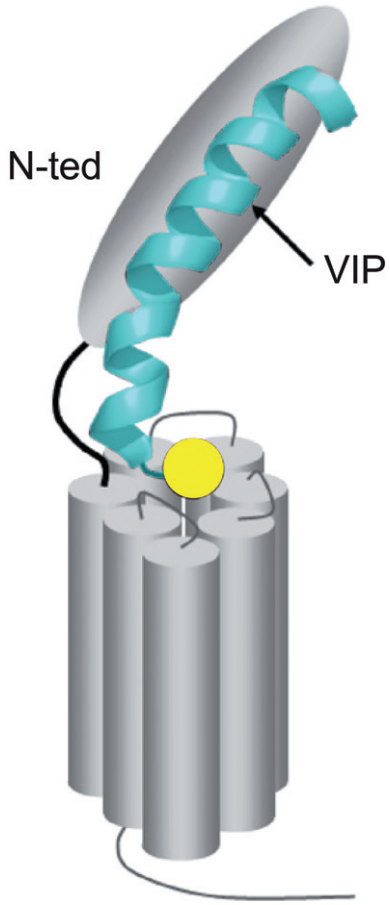

Figure 2

Schematic model of $VPAC_1$ receptor activation. The receptor N-ted traps the central and C-terminal parts (6–28) of VIP (shown in blue) and positions the N-terminal part (1–5) of VIP (yellow circle) in the receptor core for activation (adapted from Laburthe *et al.*, 2007).

et al., 2004), the $VPAC_2$ receptor (Asnicar *et al.*, 2002; Harmar *et al.*, 2002), the $VPAC_1$ receptor (Fabricius *et al.*, 2011) and the PAC_1 receptor (Hannibal *et al.*, 2001; Jongsma *et al.*, 2001; Otto *et al.*, 2001b) has permitted experimental validation of a number of physiological functions for these receptors.

Both VIP and PACAP play roles in the control of circadian rhythms in the brain's 'master clock' in the SCN of the hypothalamus. Light entrains the SCN clock through a population of retinal ganglion cells that project to the SCN via the retinohypothalamic tract and contain both glutamate and PACAP. Studies of knockout mice lacking the PAC_1 receptor or its ligand PACAP (Hannibal *et al.*, 2001; 2008; Kawaguchi *et al.*, 2003; Colwell *et al.*, 2004) show that PACAP plays a role in modulating the light-induced resetting of the behavioural rhythm and light-induced clock gene expression and physiology in the SCN. In contrast, VIP is synthesized in a population of SCN neurones, many of which are thought to receive a direct retinal innervation, and acts on $VPAC_2$ receptors, which are expressed throughout the SCN. Studies of knockout mice lacking the $VPAC_2$ receptor indicate that this receptor is necessary for the generation of normal circadian rhythms of electrical activity, clock gene expression, physiology and behaviour (Harmar *et al.*, 2002; Cutler *et al.*, 2003; Hughes *et al.*, 2004; Aton *et al.*, 2005; Bechtold *et al.*, 2008; Hannibal *et al.*, 2011). VIP-deficient mice also display a severely disrupted circadian phenotype, sharing many common features with that of $VPAC_2$ receptor null mice (Colwell *et al.*, 2003; Aton *et al.*, 2005).

Studies on PAC_1 receptor knockout mice point to a role for presynaptic PAC_1-mediated signalling at the mossy fibre synapse in long-term potentiation (LTP) and hippocampus-dependent associative learning (Otto *et al.*, 2001a; Matsuyama *et al.*, 2003). The PAC_1 receptor is also expressed in brain areas implicated in the emotional control of behaviour, such as the amygdala, bed nucleus of the stria terminalis (BNST), hypothalamus, locus coeruleus and periaqueductal grey. Consistent with this, PACAP and PAC_1 receptors are up-regulated in the BNST following chronic stress, and heightened BNST PACAP signalling produces anxiogenic behavioural responses (Hammack *et al.*, 2010). PACAP and PAC_1 receptor null mice demonstrate reduced anxiety behaviour and mice with a ubiquitous but not with a forebrain-specific deletion of the PAC_1 receptor exhibited elevated locomotor activity with strongly reduced anxiety-like behaviour (Otto *et al.*, 2001a; Matsuyama *et al.*, 2003). Furthermore, the glucocorticoid response in PACAP null animals is altered after emotional stressors (Stroth and Eiden, 2010; Tsukiyama *et al.*, 2011). PAC_1 receptor signalling in the CNS also alters feeding behaviour (Hawke *et al.*, 2009; Mounien *et al.*, 2009); PAC_1 signalling decreases food intake and promotes anorexic-like responses, which may be related to enhanced anxiety. An intronic single nucleotide polymorphism in a putative oestrogen response element within the PAC_1 receptor gene (*ADCYAP1R1*) has been associated with post-traumatic stress disorder in the female population (Ressler *et al.*, 2011), consistent with evidence that stress and oestrogen regulate the expression of the gene in animal models.

There is clear evidence that PACAP exerts neurotrophic activities during development and may prevent brain damage provoked by various types of injury. PACAP and its receptors are expressed actively in the CNS during development (Basille *et al.*, 1993; 1994). In particular, high concentrations of PAC_1 receptors are found in the external granule cell layer of the rodent cerebellum during the first two postnatal weeks (Zhou *et al.*, 1999; Basille *et al.*, 2000), a period of intense multiplication and migration of granule cells. Treatment of cultured granule cells with PACAP enhances cell survival and stimulates neurite outgrowth (Cavallaro *et al.*, 1996; Gonzalez *et al.*, 1997; Kienlen Campard *et al.*, 1997). The neurotrophic effect of PACAP is mediated through two distinct mechanisms, that is, activation of the adenylyl cyclase and PLC pathways leads to inhibition of caspase-3 activity and promotion of cell survival (Vaudry *et al.*, 2000), whereas activation of the adenylyl cyclase and MAPK pathways regulates gene expression and causes differentiation of granule neurons (Villalba *et al.*, 1997; Vaudry *et al.*, 1998; 1999). Injection of PACAP at the surface of the cerebellum of rat pups augments the number of migrating granule cells and increases the thickness of the internal granule cell layer (Vaudry *et al.*, 1999), suggesting that PACAP is a potent inhibitor of apoptosis in the cerebellum during the development. In adult animals, PACAP reduces the severity of injury in models of

focal cerebral (Ohtaki *et al.*, 2006) and retinal (Szabadfi *et al.*, 2012) ischaemia. *In vitro* PACAP also exerts a neuroprotective effect on cerebellar neurons against apoptotic cell death induced by ethanol (Vaudry *et al.*, 2002b), cisplatin (Aubert *et al.*, 2008), ceramides (Vaudry *et al.*, 2003) and oxidative stress (Vaudry *et al.*, 2002a).

VIP is also thought to play a role in neurodevelopment and in neuroprotection following injury to the CNS. For example, VIP has been shown to be protective against excitotoxin-induced white matter lesions in neonatal mice (Gressens *et al.*, 1997; 1999; Rangon *et al.*, 2005), probably acting through $VPAC_2$ receptors. $VPAC_2$ receptors have also been implicated in the control of astrocyte proliferation (Zupan *et al.*, 1998). $VPAC_2$ receptors have also been implicated in the VIP-induced expression of the neuroprotective protein activity-dependent neuroprotective protein (ADNP) in astrocytes (Zusev and Gozes, 2004) and NAP (davunetide), an active fragment of ADNP, is in clinical development for the treatment of neurodegenerative disorders (Gozes, 2011). In studies of post-natal hippocampus *in vitro*, $VPAC_2$ receptor activation was found to expand the pool of neural stem/progenitor cells by preventing either a neuronal or glial fate choice and by supporting their survival, whereas selective $VPAC_1$ receptor activation promoted a neurogenic granule cell fate (Zaben *et al.*, 2009). Two recent publications from independent groups have found associations between copy number variation in the gene encoding the $VPAC_2$ receptor and susceptibility to schizophrenia (Levinson *et al.*, 2011; Vacic *et al.*, 2011). These findings have generated some excitement in the field because they may imply that the $VPAC_2$ receptor is a potential target for the development of new antipsychotic drugs (Piggins, 2011).

Functions of VIP and PACAP receptors in the immune system

VIP and PACAP play important roles in the control of immunity and inflammation. PAC_1 receptor mRNA is constitutively expressed in macrophages and monocytes. PACAP, acting through the PAC_1 receptor appears to be protective against endotoxin-induced septic shock, acting at least in part by attenuating lipopolysaccharide-induced production of proinflammatory IL-6 (Martinez *et al.*, 2002). VIP has potent effects in the immune system, influencing T cell differentiation and migration and modulating the production of cytokines by the two subsets of mouse helper T cells: T helper 1 (Th1) cells, which mediate classical delayed-type cellular immunity and T helper 2 (Th2) cells, which mediate hypersensitivity reactions, such as allergy. $VPAC_1$ receptors are highly expressed constitutively on T cells, especially Th cells, whereas $VPAC_2$ receptors are expressed marginally or not at all by unstimulated Th cells but are up-regulated to high levels by Th cell stimulation. Studies on $VPAC_2$ receptor knockout mice (Goetzl *et al.*, 2001; Voice *et al.*, 2002) and on transgenic mice overexpressing the $VPAC_2$ receptor in CD4 T cells (Voice *et al.*, 2001; 2002; 2003) suggest that the receptor regulates the balance between Th1 and Th2 by stimulating production of more Th2-type cytokines, due to expansion of the Th2-type subset. PACAP knockout mice exhibited the predicted hyperinflammatory response in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, with an enhanced Th1/Th2 cytokine profile, but also with a reduced expansion of regulatory T cells (Tan *et al.*, 2009). VIP-deficient mice, on the other hand, exhibited a paradoxical resistance to EAE, with a failed entry of inflammatory cells into the CNS parenchyma (Abad *et al.*, 2010), pointing to a critical role for VIP in T cell trafficking. VIP receptor agonists and antagonists may have therapeutic potential in the treatment of inflammatory and autoimmune diseases such as Crohn's disease (Abad *et al.*, 2003), rheumatoid arthritis (Juarranz *et al.*, 2004) and multiple sclerosis (Gonzalez-Rey *et al.*, 2006).

Functions in the gastrointestinal tract

PACAP and VIP and their receptors are expressed widely in the gastrointestinal tract. In the gastric mucosa, PACAP-containing enteric nerve fibres have been described, which are co-localized with PAC_1 receptors (Miampamba *et al.*, 2002). PACAP appears to have diverse functions in the stomach that depend on whether the cell target expresses either PAC_1 or $VPAC_1$ receptors. PAC_1 is expressed on gastric enterochromaffin-like (ECL) cells and involved in the regulation of gastric acid secretion, whereas $VPAC_1$ is expressed by the somatostatin containing gastric D cells and thought to inhibit gastric acid secretion. In the rat stomach, PACAP released by enteric neurons innervating the mucosa appears to mediate the nocturnal increase in gastric acid secretion (Zeng *et al.*, 1999). VIP knockout mice exhibit a reduction in gastrointestinal motility similar to that observed in patients with Hirschsprung's disease (Lelievre *et al.*, 2007). These observations suggest that PACAP, VIP and their receptors appear to play an important role in the regulation of gastrointestinal function and underscore the importance of further studies in this area.

Relevance to cancer

The best-known association of VIP with cancer is the 'watery diarrhoea syndrome' (also known as 'pancreatic cholera' or Verner Morrison syndrome) caused by the ectopic secretion of VIP by some tumours (VIPomas), usually of non-β pancreatic islet cell origin (Modlin *et al.*, 1978). The symptoms of this condition are thought to result from the action of VIP on $VPAC_1$ receptors in the intestinal mucosa to stimulate chloride secretion and water movement into the intestinal lumen, an effect mimicked by selective $VPAC_1$ receptor agonists in animal models (Tsutsumi *et al.*, 2002).

Most of the commonly occurring human tumours express $VPAC_1$ receptors (Reubi *et al.*, 2000). $VPAC_2$ expressing tumours are much rarer: they include a high proportion of gastrointestinal stromal tumours (Reubi *et al.*, 2004) and also leiomyomata (benign smooth muscle neoplasms, e.g. uterine fibroids). Consistent with the role of PACAP in the CNS and the sympathoadrenal system, PAC_1 receptors are often expressed in tumours of neuroectodermal origin (e.g. neuroblastoma, glioma, phaeochromocytoma and pituitary adenoma; Robberecht *et al.*, 1993; 1994; Vertongen *et al.*,

1996) as well as in endometrial carcinoma (Reubi *et al.*, 2000). The use of radioactive ligands of VIP and PACAP receptors for imaging or therapy of tumours has met with limited success, perhaps due to difficulties in achieving selectivity for tumour tissue over normal tissues and in identifying analogues with appropriate pharmacokinetic properties. Numerous cell lines derived from tumours have been found to express receptors for VIP and PACAP (see http://www.tumor-gene.org/GPCR/gpcr.html) and have provided useful tools for the study of receptor function. For example, the first report of a receptor with $VPAC_2$ pharmacology used SUP-T1 lymphoblasts (Robberecht *et al.*, 1988) and PC12 phaeochromocytoma cells have been used extensively to study the effects of PACAP on cell survival, cell proliferation, neurite outgrowth and the underlying signalling pathways (Vaudry *et al.*, 2009).

Other functions in the periphery

VIP and PACAP, acting through PAC_1 and $VPAC_2$ receptors on pancreatic β-cells, have been implicated in the control of pancreatic insulin secretion. PAC_1 receptor-deficient mice display impaired insulinotropic response to glucose, reduced glucose tolerance and impaired glucagon response to insulin-induced hypoglycaemia (Jamen *et al.*, 2000; Persson and Ahren, 2002) and overexpression of PACAP in mouse pancreatic β-cells has been reported to enhance insulin secretion and ameliorate streptozotocin-induced diabetes (Yamamoto *et al.*, 2003) and to inhibit hyperinsulinemia and islet hyperplasia in agouti yellow mice (Tomimoto *et al.*, 2004). $VPAC_2$ receptor null mice have been reported to be able to maintain a normal response to glucose challenge with lower levels of insulin than wild-type mice, suggesting a significant increase in insulin sensitivity in the knockout mice (Asnicar *et al.*, 2002). A selective peptide agonist of the $VPAC_2$ receptor stimulated glucose-dependent insulin secretion in isolated rat and human pancreatic islets, increased insulin synthesis in purified rat islets and caused a dose-dependent increase in plasma insulin levels in fasted rats, suggesting that $VPAC_2$ receptor agonists may be a useful therapy for the treatment of type 2 diabetes (Tsutsumi *et al.*, 2002).

Consistent with the expression of PAC_1 and $VPAC_1$ receptors in adrenomedullar cells (Usdin *et al.*, 1994; Moller and Sundler, 1996; Yon *et al.*, 1998; Shioda *et al.*, 2000), PACAP and VIP are potent activators of catecholamine release *in vitro* (Cheung and Holzwarth, 1986; Watanabe *et al.*, 1992) and *in vivo* (Lamouche *et al.*, 1999). Interestingly, PACAP strongly increases VIP mRNA expression in bovine chromaffin cells (Lee *et al.*, 1999). Intravenous administration of PACAP enhances the secretion of corticosteroids in dog (Kawai *et al.*, 1994) and calf (Edwards and Jones, 1994). In human, PACAP-induced stimulation of cortisol secretion from adrenal slices is suppressed by the β-adrenoceptor antagonists (Neri *et al.*, 1996; Breault *et al.*, 2000) suggesting that the effect of PACAP on corticosteroid secretion is mediated through its stimulatory action on catecholamine release.

The presence of PACAP in primary sensory neurones and the PAC_1 receptor in the dorsal horn of the spinal cord (Jongsma *et al.*, 2000) suggest a role for the PAC_1 receptor in pain responses. PAC_1 receptor knockout mice displayed impaired nociceptive responses to chemical, thermal and mechanical stimuli (Jongsma *et al.*, 2001) and PACAP-deficient mice also displayed abnormal pain responses (Mabuchi *et al.*, 2004).

Although most studies of PAC_1 receptor knockout mice have found these animals to be superficially normal and viable, it has been reported that when crossed onto a C57BL/6 background, almost all PAC_1 receptor knockout mice developed pulmonary hypertension and right heart failure after birth, suggesting an important role for PAC_1-mediated signalling for the maintenance of normal pulmonary vascular tone during early postnatal life (Otto *et al.*, 2004).

Perspectives

VIP and PACAP play diverse and important roles in the CNS, with functions in the control of circadian rhythms, learning and memory, anxiety and responses to stress and brain injury. The development of drugs acting on these receptors may lead to new treatments for sleep disorders, stroke, neurodegenerative disorders and age-related memory impairment. Genetic studies also implicate the $VPAC_2$ receptor as a potential target for the development of new antipsychotic drugs and suggest involvement of the PAC_1 receptor in post-traumatic stress disorder. The role of VIP and PACAP in the gastrointestinal tract suggest that these hormones and their receptors would provide a therapeutic target for the management of gastric acid secretory disorders and disorders affecting gastrointestinal motility such as functional bowel syndromes. The involvement of VIP and PACAP in immune function suggests potential applications in the treatment of diseases such as Crohn's disease, rheumatoid arthritis and multiple sclerosis. Other peripheral functions of VIP and PACAP indicate potential importance in diabetes, pain and hypertension. The major impediment to translational research on VIP and PACAP is that all of the currently useful pharmacological tools are peptides. The first small molecule antagonists of PAC_1 receptors (Beebe *et al.*, 2008) and human $VPAC_2$ receptors (Chu *et al.*, 2010) have been described recently and may herald important breakthroughs in understanding the therapeutic opportunities offered by these receptors.

Acknowledgements

AJH is supported by IUPHAR, the British Pharmacological Society and the British Heart Foundation Centre of Research Excellence Award (RE/08/001).

Conflicts of interest

Prof. Illana Gozes serves as a Director, Chief Scientific Officer at Allon Therapeutics Inc. clinically developing davunetide (NAP).

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